Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore

Synopsis

on

Qualitative and Quantitative analysis of Proteins and Lipids in Soybean (*Glycine max L. Merril* cultivar 9560) after pre-sowing treatment of seeds with static magnetic field.

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Submitted by

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"Qualitative and Quantitative analysis of Proteins and Lipids in Soybean Glycine max L. Merril (cultivar 9560) after pre-sowing treatment of seeds with static magnetic field."

1. Introduction

In the global scenario, legumes are known for the fulfilment of nutritional purposes. Among all of them Soybean (Glycine max L. Merril) is the most significant seed legume in the world. About 25% of the global need of edible oil and almost two-thirds of the protein concentrate used in cattle feed is supplemented by soybean crop (Hartman et al., 2011). The meal produced by Soybean has been served as an important component in formula feeds for fish and poultry. It is supposed that Cultivation and consumption of Soybean is as old as the Chinese agrarian era (Yin et al., 2011). According to Michelfelder, (2009) Soybean protein is referred as a complete protein because of its amino acids composition. A wide history of nutraceutical uses of soybean protein for preventing diabetes and heart disease has been documented so far. In present time, globally three countries USA, Brazil, and Argentina are considered as "Big 3" producers of soybean. The versatile utilities of Soybean were lately recognized in the West. It was estimated that only China itself produced about 80% of the world's soybean in past two decades. Introduction of soybean in India was supposed in the 10th century AD, when voyaging traders from Indonesia traders who brought soybeans with them while travelling through Myanmar and Himalays (Shurtleff and Aoyagi, 2010). In addition, it has been proposed that the Indian subcontinent acts as the crop's secondary domestication centre after China (Agarwal et al., 2013). Currently India is ranked 5th after the United States, Brazil, Argentina, and China, in terms of soybean cultivation area and yield output. According to Agarwal et al., (2013) and Waha et al., (2020) India contributes 10% of the world's soybean land, but just 4% of the world's soybean grain, demonstrating India's low crop yield (1.1 t/ha) in comparison to other competitors (global average: 2.2 t/ha).

4/8/23

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1. Introduction

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According to **Michelfelder, (2009)** Soybean protein is referred as a complete protein because of its amino acids composition. A wide history of nutraceutical uses of soybean protein for preventing diabetes and heart disease has been documented so far. In present time, globally three countries USA, Brazil, and Argentina are considered as "Big 3" producers of soybean. The versatile utilities of Soybean were lately recognized in the West. It was estimated that only China itself produced about 80% of the world's soybean in past two decades. Introduction of soybean in India was supposed in the 10th century AD, when voyaging traders from Indonesia traders who brought soybeans with them while travelling through Myanmar and Himalayas **(Shurtleff and Aoyagi, 2010)**. In addition, it has been proposed that the Indian subcontinent acts as the crop's secondary domestication centre after China **(Agarwal et al., 2013)**. Currently India is ranked 5th after the United States, Brazil, Argentina, and China, in terms of soybean cultivation area and yield output. According to **Agarwal *et al.*, (2013)** and **Waha *et al.*, (2020)** India contributes 10% of the world's soybean land, but just 4% of the world's soybean grain, demonstrating India's low crop yield (1.1 ton/hectare) in comparison to other competitors (globalaverage: 2.2 ton/hectare).

It is well known that soybean is an oil seed that contains a variety of beneficial components, such as protein, carbohydrates, vitamins, and minerals. An average dried soybean contains 5% minerals, 36% protein, 19% oil, 35% carbs (17% from dietary fibre) and several other elements, including vitamins **(Banaszkiewicz, 2011)**. **Friedman and Brandon (2001)** stated that soy protein is one of the cheapest forms dietary proteins.

Since soybean proteins contain the majority of the essential amino acids needed for both animal and human nutrition, they are thought to be a good substitute for animal protein. Their nutritional profile, with the exception of sulphur amino acids (methionine and cysteine), is almost identical to that of animal protein.

The agronomic use of magnetic field (MF) in plants has shown promise in modifying traditional plant production systems, increasing mean germination rates, root and shoot growth, having high productivity, increasing photosynthetic pigment content, intensifying cell division, as well as water and nutrient uptake **(Kisvarga *et al.*, 2022).**

According to **Araujo *et al.*, (2016)** in the past ten years, plant science has made significant progress regarding the impacts of the MF in seeds and plants. There is solid evidence to support the idea that magnetic pre-germination treatment before sowing seeds can lower planting expenses by significantly increasing germination rates and promoting plant growth. Others, however, demonstrated that development is impeded.

The use of chemicals (such as hormones) in the pre-sowing seed treatment increases crop yields overall and homogeneity, despite the fact that they are seen to be very effective, intrusive, environmentally inappropriate, and challenging to use **(Zulfiqar, 2021)**.

In recent decades, the discovery of hormones and chemical fertilizers played a significant role in increasing crop yields. However, the use of chemical fertilizers and other materials is currently contested, which has led to the use of less expensive, environmentally friendly alternatives like gamma rays, laser, electron beam, microwave, Magnetic field, and radiofrequency energies to bio-stimulate seeds. Therefore, biophysical treatment may be thought of as an option because it decreases the quantity of toxin in plants or plant-derived products, boosting the safety of food and the environment **(Sarraf *et al.*, 2020)**.

In the agricultural industry, treating seedlings with magnetic fields has gained a lot of popularity. The practice of "magnetopriming," which involves dry seed priming and pre-treatment with MF, has been shown to boost numerous crops' rates of germination and seedling vigour. Numerous studies have examined the metabolic modifications brought on by magnetopriming in non-stressful conditions during seed germination

(Hasanuzzaman and Fotopoulos, 2019). According to **Maffei, (2014)** another benefit of using magnetic field is its potential to promote membrane permeability and boost germination rates, which will make it easier for seeds to absorb water. Beyond increasing germination rates, MF-exposed seeds have other advantageous impacts like improved cell proliferation potential, which could lead to plants growing quickly.

Amino acids, proteins, fatty acids and lipids are major nutritive biochemical entities included as a dormant seed. Physical and chemical methods of seed treatment may alter the embedded values of the aforesaid nutritive components. Pre treatment of seed of some economically important crops such as Sunflower, Amaranthus, Maize, Rice, Wheat and Soybean with either static or pulsed magnetic field have been reported for the alteration in the storage protein and lipid profile **(Radhakrishnan, 2018)**.

Since Soybean is a very important crop from the economic, agronomic and nutritive point of view, several agronomical techniques have been adopted to develop improved variety of the same for certain purpose like enhanced protein values, fatty acid content, oil content, stress tolerance etc. Above mentioned text reflects that magnetic field treatment of seed is one of the important physical techniques brought about in the crop improvement strategies. So that, proposed study will focus on the **"Qualitative and Quantitative analysis of Proteins and Lipids in Soybean *Glycine max* L. Merril (cultivar 9560) after pre-sowing treatment of seeds with static magnetic field."**

2. Review of literature

Pre-sowing chemical or physical treatments that break seed dormancy increase seed germination, protect seeds from pests and diseases, and ensure consistent crop stand development in the field. Due to degradation, seeds lose their vigour and vitality while in storage, which ultimately leads to the loss of expensive seed stock. Pre-sowing methods that hydrate the seeds throughout the treatment process, such as osmo-priming, hydro-priming, halo-priming, and solid matrix priming, improve germination at the expense of seed storage **(Halmer. 2006)**.

According to **Lutts *et al.*, 2016** such seeds must be sowed right away after the treatment because they cannot be kept for longer periods of time. Considering this limitation, priming the seed with a magnetic field (MF) might be a workable substitute for enhancing seed vigour.

Due to the magnetic field's heavy usage over the past few decades, the environment for humans has changed. Plants are more exposed to MF than any other species due to the excessive and continuous use of domestic appliances, medical equipment, transit vehicles, and communication equipment (**Vian *et al.*, 2016**).

In the past ten years, plant science has made significant progress regarding the impacts of the MF in seeds and plants. There is solid evidence to support the idea that pre-treating seeds with magnets before sowing can significantly enhance germination rates while also promoting plant growth, which lowers planting expenses **(Nyakane *et al.*, 2019)**.

Kataria *et al.*, 2015 demonstrated magnetopriming of soybean and maize seeds with static magnetic field of 200 mT for 1 hour and with resulted improvement in germination related parameters like germination-percentage, water uptake, seedling length, fresh and dry weight of seedlings and vigor indices, increased activity of amylase and protease.

Rare are the reports on harvested seeds' nutritional alterations brought on by MF pretreatment. According to the findings, MF stimulated globulin formation while suppressing prolamin production. However, lipid content in seeds increased as a result of

MF exposure since these fatty acids-caprylic acid, palmitic acid, heptadecanoic acid, linoleic acid, lignoceric acid, and eicosatetraenoic acid, all rose in greater concentration as compared with the control **(Radhakrishnan, 2018).**

According to a field experiment carried out by **Guruprasad *et al.*, in 2023** to determine the effects of magneto-priming soybean var. JS-335 plants. Results indicated that treatments with static magnetic fields of 150 mT and 200 mT for one hour each improved growth (shoot and root growth parameters), carbon fixation (PSII efficiency, gas exchange parameters, carbonic anhydrase activity), and nitrogen fixation (leghemoglobin content, total protein content, nitrate reductase activity).Additionally, decreased levels of antioxidant enzymes, MDA (malondialdehyde), and ASA (Ascorbic Acid (reduced form of ascorbate) show that magneto-priming reduces oxidative stress in SMF-primed soybean plants.

According to a study by **Guruprasad *et al.*, 2012** when seeds were exposed to a magnetic field, the formation of ROS, which is mediated by cell wall peroxidase, increased while ascorbic acid concentration, superoxide dismutase activity, and ascorbate peroxidase activity decreased in the hypocotyl of germination seeds.
Static magnetic fields (SMF) were used to treat maize (Zea mays var: Ganga safed) seeds, using a 200 mT magnetic field strength. When the seeds were germinated, SMF dramatically increased the activity of the á-amylase enzyme relative to the untreated seeds **(Guruprasad *et al.*, 2018)**.

It was also investigated how well magnetic bio stimulation affected pre-sown sunflower seeds, seedling growth metrics (such as biomass, root and shoot length, fresh and dry weight of roots, shoots, and leaves, and plant height), and antioxidant activity. The mean germination growth (100 ± 0.02) and final emergence rate were among the growth parameters that were significantly affected by the magnetic treatment at 50 mT/45 min. In terms of the antioxidant characteristics, seed variety FH620 at a concentration of 500 g/L demonstrated noteworthy results in comparison to other variations **(Bukhari *et al.*, 2021).**

Harb *et al.*, 2021 experimentally assessed that, static magnetic fields were applied to lentil seedlings in a systematic manner from low intensity to high intensity. The

biochemical alterations in lentil seedlings were then evaluated in relation to the successful magnetic treatments that encouraged or hindered growth. First, static magnetic fields of 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mT were applied to lentil seeds for 5, 10, 15, 20, 25, and 30 minutes, respectively. The morphological alterations in lentil seedlings were then evaluated. The effects of several magnetic treatments on lipid peroxidation and the activity of a few antioxidant enzymes in lentil seedlings were examined. Although there was no significant increase in lipid peroxidation following the 20 mT for 25 min magnetic treatment, catalase and superoxide dismutase activity increased significantly (0.57 and 1.09 units/mg protein compared to 0.15 and 0.40 units/mg protein for the control, respectively). Therefore, priming lentil seeds with the appropriate magnetic treatment could be an effective, cost-effective, and environmentally friendly method for enhancing lentil growth.

3. Hypothesis

As stated in above literature, there are two chief aspects of soybean seeds have been studied, the first is qualitative analysis and the second objective is quantitative analysis of both proteins and lipids. Both these parameters are directly associated with the crop yield and productivity **Kataria *et al.*, 2015**. To enhance the aforesaid parameters magnetic field treatment is a very effective and ecofriendly technique **Guruprasad *et al.*, in 2023**. Hypothesis to undertake proposed study is that, how much alteration in germination efficiency, content of some beneficial enzymes,amino acids and lipid profile occurs when seeds will be exposed to the variable dosage of static magnetic field *viz.* 50 to 200 MT.

4. Objectives of the study

The specific objectives of the proposed study will be as follows:

- Assessment of germination efficiencies of Soybean seed exposed to static magnetic field *viz.* 50 to 200 MT.
- Assessment of Protease activity in germinated Soybean seeds.
- Assessment of Lipase activity in germinated Soybean seeds.

- Analysis of growth and yield of Soybean plants exposed to static magnetic field *viz.* 50 to 200 MT.
- Qualitative and Quantitative estimation of Proteins in F1 generation obtained after harvest from the plants exposed to static magnetic field.
- Qualitative and Quantitative estimation of Lipids in F1 generation obtained after harvest from the plants exposed to static magnetic field.

5. Methodology

1. Seed treatment: Soybean seeds cultivar 9560 will be treated with static magnetic field in variable dosage viz. 50, 100, 150 and 200 MT as per the method of **Shine and Guruprasad (2012)**.

2. Seed germination: Seed germination efficiency will be assessed as per the standard procedure of **Ijaz *et al.*, 2012.**

3. Assessment of Protease activity will be estimated ingerminated Soybean seedsas per the standard procedure by **Kunitz (1947)**.

4. Assessment of Lipase activity will be estimated in germinated Soybean seeds as per the standard procedure by **A. Sadasivan and A. Manickam (1992).**

5. Qualitative and quantitative estimation of Proteins:

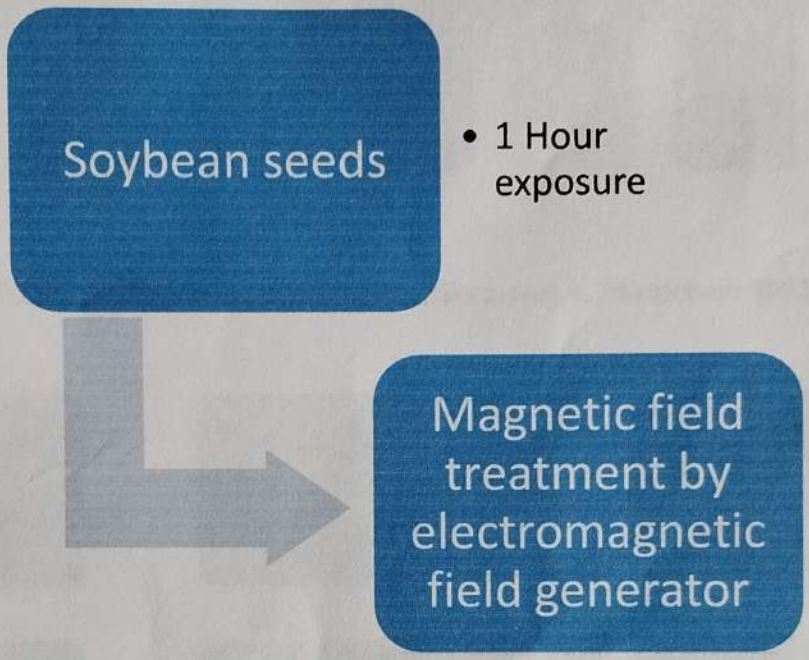
Protein extraction to be done by the method of **Kaur and Singh (2007)** and quantification of extracted total protein to carried out using **Folin phenol method by Lowery *et al.*, 1951.**

Protein analysis to be done by using SDS-PAGE and 2D-Electrophoresis by **Oddepally *et al.*, 2013.** SDS-PAGE will be done to identify if any new band of protein is present or not.

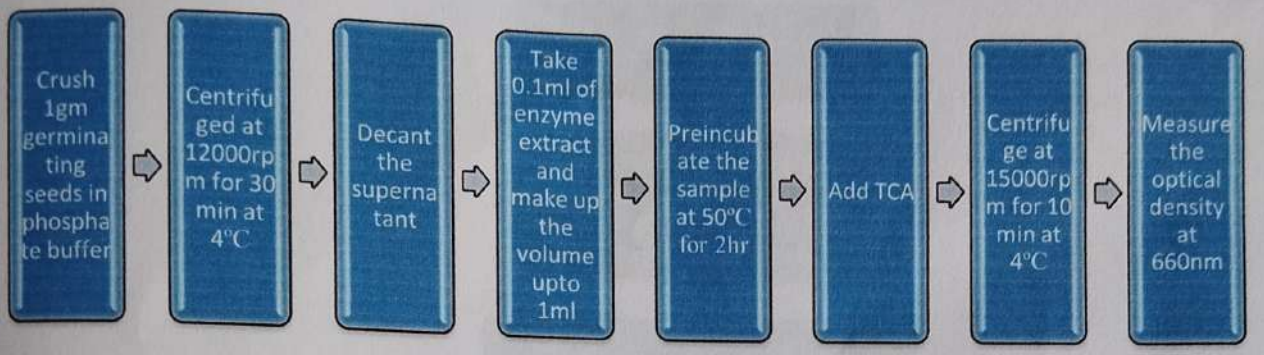
6. Qualitative and quantitative estimation of Fatty acids:

Fatty acids estimation to be done by using gas chromatography done by **Bertrand and Ludger (2001).**

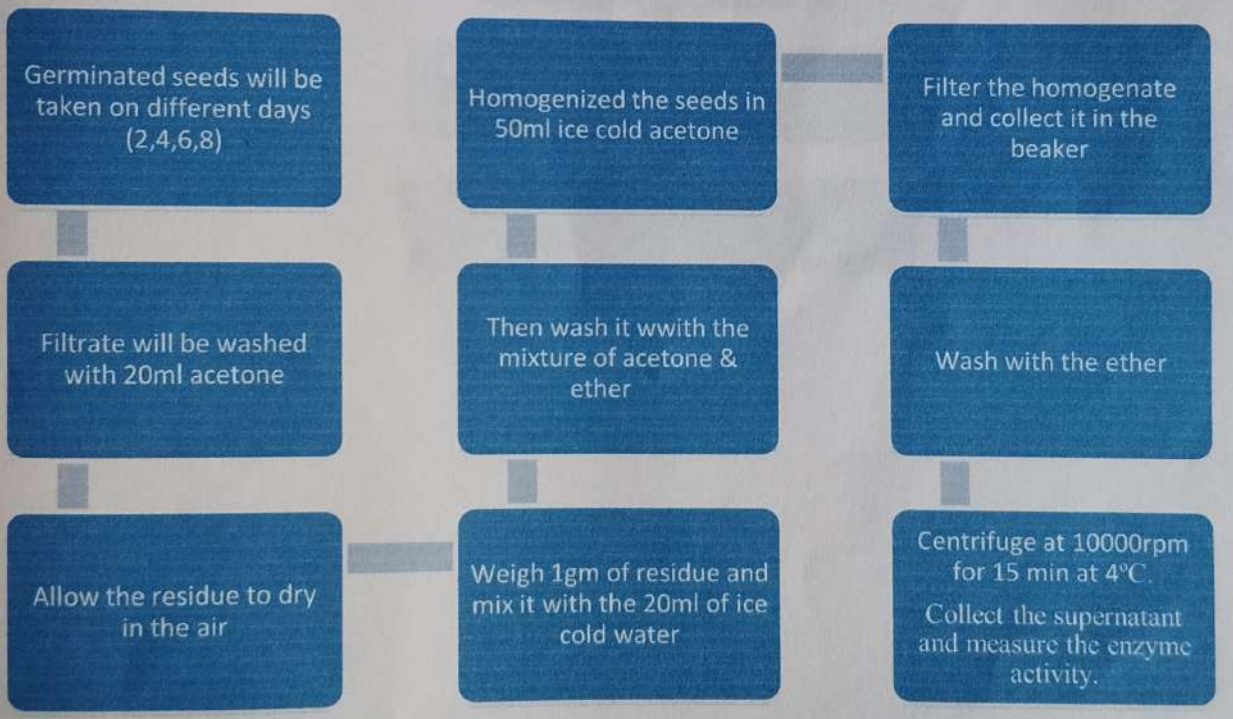
Method 1: Seed Treatment with static magnetic field (Shine and Guruprasad 2012)



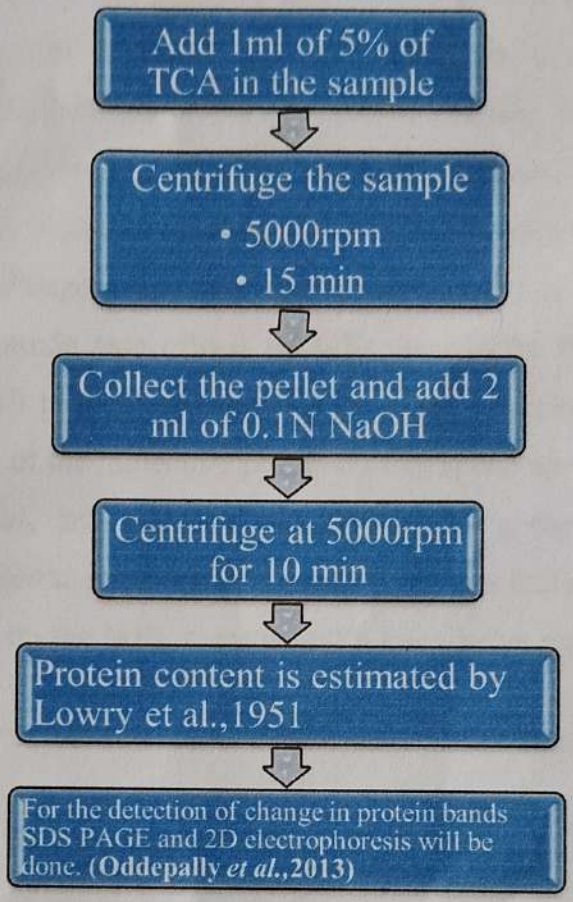
Method 2: Protease activity (Kunitz 1947)


Crush 1gm germinating seeds in phosphate buffer
Centrifuged at 12000rpm for 30 min at 4°C
Decant the supernatant
Take 0.1ml of enzyme extract and make up the volume upto 1ml
Preincubate the sample at 50°C for 2hr
Add TCA
Centrifuge at 15000rpm for 10 min at 4°C
Measure the optical density at 660nm

Method 3: Lipase activity (A. Sadasivan and A. Manickam 1992)


Germinated seeds will be taken on different days (2,4,6,8)
Homogenized the seeds in 50ml ice cold acetone
Filter the homogenate and collect it in the beaker
Filtrate will be washed with 20ml acetone
Then wash it wwith the mixture of acetone & ether
Wash with the ether
Allow the residue to dry in the air
Weigh 1gm of residue and mix it with the 20ml of ice cold water
Centrifuge at 10000rpm for 15 min at 4°C.
Collect the supernatant and measure the enzyme activity.

Method 4: Protein Estimation by (Lowery *et al.*, 1951)


Add 1ml of 5% of TCA in the sample
Centrifuge the sample
• 5000rpm
• 15 min
Collect the pellet and add 2 ml of 0.1N NaOH
Centrifuge at 5000rpm for 10 min
Protein content is estimated by Lowry et al.,1951
For the detection of change in protein bands SDS PAGE and 2D electrophoresis will be done. (Oddepally et al.,2013)

6. Future scope of this study

A suitable ratio of MF intensity to exposure time is necessary to improve seed germination, crop productivity, and development. Numerous studies have demonstrated that its beneficial effects can enhance seed germination, root and shoot length, absorption of water and CO_2, pigment content in photosynthetic organisms, and ultimately the growth of agricultural output even in the presence of abiotic challenges. However, both this element of magneto biology and the potential genotoxic side effects of MFs need to be further investigated. These results have all brought attention to the need for more research to deepen our understanding of the molecular processes that speed up seed germination, increase seedling vigour, and increase the photosynthetic capacity of magneto-primed plants under abiotic stressors. In overall, it appears that despite all of the work and research done on the MFs, there is still a knowledge gap, necessitating additional tests.

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